electrophoresed in 10% SDS-polyacrylamide gels imbedded with 0.25 mg/mL of MBP in the separating gel. After protein renaturation, the kinase reaction was carried out as described in Materials and Methods. The sizes of activated kinases are given in kilodaltons. **Fig. 1B:** The activities of 48-kD kinase (in TMV- [●] and mock- [○] inoculated leaves) and 44-kD kinase (in TMV- [▲] and mock- [△] inoculated leaves) were quantitated using a PhosphorImager and the relative activities were plotted against time. Kinase activities were normalized to the level present at the zero time point for the 48-kD kinase, which was given a value of 1.

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(Page 6, Line 17) **Figures 2A-2C.** Immuno-complex kinase assays using sequence-specific antibodies against SIPK and WIPK. **Fig. 2A:** An antibody raised against a peptide (p44N) corresponding to the unique N-terminus of WIPK, Ab-p44N, specifically recognized the WIPK protein. Two nanograms each of recombinant HisSIPK, HisNtf4, HisWIPK, and HisNtMPK6 or 20 μg of protein extracts from 48 hr mock- or TMV-inoculated tobacco leaves (maintained throughout infection at 22°C) were subjected to immunoblot analysis with Ab-p44N in the absence or presence of 0.2 μg/mL competitor peptides p44N or p48N. **Fig. 2B:** Immuno- complex kinase assay of TMV-activated kinase using SIPK-specific antibody, Ab-p48N. Protein extracts (50 μg) from TMV- or mock-inoculated leaf tissue were reacted with Ab-p48N (2.5 μg). The resultant antigen-antibody complex were precipitated with protein A-agarose beads, washed extensively before addition to a kinase assay mixture with [γ-^{32}P]-ATP and MBP as substrates. The reaction mixture, including the phosphorylated MBP, were then fractionated by SDS-PAGE. **Fig. 2C:** Immuno-complex kinase assay of TMV-activated kinase using WIPK-specific antibody, Ab-p44N. Protein extracts (50 μg) from TMV- or mock-inoculated leaves were immunoprecipitated with Ab-p44N (2.5 μg) and the kinase activity of the immuno-complex was determined as above. Times in B and C are

given in hps from 32°C to 22°C.

(Page 7, Line 9) **Figures 3A and 3B.** Activation of *WIPK* gene expression by TMV in tobacco plants (cv Xanthi nc [NN]) after temperature shift. **Fig. 3A:** Increase in steady-state levels of *WIPK* mRNA in TMV-infected plants. Duplicates of leaf discs used in Figure 1 were extracted for total RNA, thus facilitating direct comparison of the induction kinetics of mRNA and enzymatic activity. Twenty micrograms of total RNA per lane were separated on 1.2% formaldehyde-agarose gels and transferred to Zeta-probe membranes. Blots were hybridized with random primer-labeled inserts consisting of either a full-length cDNA of *WIPK* (data shown) or its 3'-untranslated region (data not shown). **Fig. 3B:** Increase of WIPK protein in TMV-infected tobacco after temperature shift. Samples containing 20 μg of protein from the leaf extracts used for Fig. 1A were separated on 10% SDS-polyacrylamide gels. After blotting to nitrocellulose, the WIPK protein was detected with Ab-p44N.

A2 cont'd

(Page 7, Line 27) **Figures 4A-4C.** Activation of WIPK by TMV in tobacco plants (cv Xanthi nc [NN]) maintained at 22°C throughout infection. **Fig. 4A:** Increase in steady-state levels of *WIPK* mRNA in TMV-infected tobacco plants. Tobacco plants were inoculated with TMV or buffer (mock) as in Figure 1 except a higher concentration of TMV was used (5 μg/mL). Leaf discs were taken at the indicated times in hr post inoculation (hpi). Total RNA was prepared and analyzed for *WIPK* mRNA as described in Figure 3. **Fig. 4B:** Increase of WIPK protein in TMV-infected tobacco maintained at 22°C. Protein extracts were prepared from duplicate leaf discs to those used in Fig. 4A. Twenty micrograms of protein was analyzed by immunoblotting using Ab-p44N as described in Figure 3. **Fig. 4C:** Induction of WIPK enzymatic activity in TMV-infected tobacco maintained at 22°C. Selected protein

extracts from (B) were analyzed by immuno-complex kinase assay using WIPK-specific Ab-p44N as described in Figure 2.

(Page 8, Line 26) **Figures 6A-6C.** TMV activation of *WIPK* transcription in tobacco is *N* gene dependent, SA independent and systemic. **Fig. 6A:** *WIPK* mRNA induction in tobacco by TMV infection is *N* gene dependent. TMV-susceptible tobacco plants (*N. tabacum* cv Xanthi [nn] which lacks *N* resistance gene) were infected and *WIPK* mRNA detected by RNA gel blot analysis. **Fig. 6B:** Induction of *WIPK* mRNA by TMV infection is SA independent. Transgenic tobacco (cv Xanthi nc [NN]) plants expressing the *NahG* gene were infected and *WIPK* mRNA determined by RNA gel blot analysis. **Fig. 6C:** Systemic induction of *WIPK* mRNA after TMV infection. Three leaves from each tobacco plants (cv Xanthi nc [NN]) were either inoculated with TMV or buffer only (mock) and maintained at 22°C. At indicated days post inoculation (dpi), leaf discs were taken from the upper uninoculated leaves. Total RNA was isolated and *WIPK* mRNA levels were determined.

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(Page 9, Line 9) **Figures 7A and 7B.** Autoradiograms of immunoblot assays showing that the 48-kD MBP kinase activated by water infiltration and wounding is encoded by *SIPK* rather than *WIPK*. Protein extracts (50 µg) from water-infiltrated, cutting or abrasion-wounded leaves were immunoprecipitated with either the SIPK-specific antibody Ab-p48N **(Fig. 7A)** or the WIPK-specific antibody Ab-p44N **(Fig. 7B)**. Kinase activity of the resultant immuno-complexes was subsequently determined as described in Example 1.

(Page 9, Line 19) **Figures 8A and 8B.** Autoradiograms of RNA or immunoblot assays showing that water infiltration and wounding induce transient increases in *WIPK* mRNA levels, but little or no increases in WIPK protein level. **Fig. 8A:** Total

RNA was extracted at the indicated times from water infiltrated or wounded leaves and subjected to RNA gel blot analysis. Blots were sequentially hybridized with the 3' UTR and then the full length *WIPK* cDNA. Both probes yielded the same result; thus, only the autoradiogram produced with the full-length cDNA is shown. **Fig. 8B:** Protein extracts (20µg) were subjected to immunoblot analysis with the WIPK-specific antibody, Ab-p44N.

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(Page 26, Line 18) Once cloned, a constitutively activated WIPK kinase may be constructed, as has been done for mammalian (Mansour et al., 1994) and *Xenopus* (Gotoh et al., 1994) MAPK kinases. MAPK kinases are activated by dual phosphorylation of a SXXXS/T motif (SEQ ID NO: 1) in the kinase subdomain VIII by MAPK kinase kinase. Substitution of these two Ser/Thr residues with Asp or Glu was found to increase basal activity about 100 fold, and cells transformed with these mutants exhibited constitutive activation of the MAPK regulated pathway (i.e. AP-1 transcription; Mansour et al., 1994). The constitutively activated mutatn WIPK will be transformed into plant cells and the corresponding transgenic plants obtained. These plants will have the WIPK-mediated signal transduction pathway constitutively activated.

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(Page 28, line 29) **Antibody production and immunoblot analysis.** The peptides p44N (MADANMGAGGGQFPDFPS; SEQ ID NO: 2) and p48N (MDGSGQQTDTMMSDAGAEQPPTAP; SEQ ID NO: 3), which correspond respectively to the unique N-termini of the WIPK and the SIPK proteins, were synthesized and conjugated to keyhole limpet hemacyanin (KLH) carrier. Polyclonal antisera were raised in rabbits and purified by affinity column chromatography (Zymed Laboratory, South San Francisco, CA).

A4

(Page 31, Line 34) **The 44-kD kinase activated by TMV is encoded by *WIPK*.** The size and substrate preference of the

A5

44-kD kinase suggested that it also might be a MAP kinase, possibly that encoded by *WIPK*. To confirm or refute this possibility, antibody was prepared in rabbits against a peptide corresponding to the unique N-terminus (p44N, MADANMGAGGGQFPDFPS; SEQ ID NO: 2) of WIPK and affinity purified. The specificity of the Ab-p44N was assessed by immunoblot analysis against a panel of different MAP kinases as described above for Ab-p48N. Ab-p44N recognized only the His-tagged WIPK protein (Fig. 2A). Addition to the immuno reaction of the competitor peptide p44N, but not the p48N, blocked binding of Ab-p44N to the His-tagged WIPK protein (Fig. 2A), further demonstrating the specificity of this antibody.

A5 cont'd

(Page 36, line 13) WIPK was originally isolated based on an increase in its mRNA level after wounding; it was presumed to encode a wounding-activated 46-kD MAP kinase (Seo et al., 1995). We have confirmed that wounding transiently induces *WIPK* at the mRNA level. However, there is little or no increase in WIPK protein following this very transient induction of *WIPK* mRNA. Furthermore, using the WIPK- and SIPK-specific antibodies Ab-p44N and Ab-p48N, respectively, we have discovered that the wounding-activated kinase is the 48-kD SIPK, not the 44-kD WIPK (data for SIPK set forth in Zhang & Klessig, 1998). In this regard it should be noted that the molecular weight of the wounding-activated MBP kinase described by Seo et al. (1995) (46-kD) and by the present inventors (44-kD) is not significantly different. Such slight differences in estimated molecular weight based on SDS-PAGE occurs commonly among different laboratories.

A6

In the Claims:

A7 Sub B1

1. (Amended) A transgenic plant expressing an N gene, having enhanced resistance to a plant disease-causing agent selected from the group consisting of tobamoviruses, elicitin-